Starch Distribution as affected by Fungi.*

By Byron D. Halsted.

A study of the distribution of starch in variegated leaves led to a consideration of the presence of this carbohydrate in leaves that were more or less affected with parasitic fungi. Thus, from the variegated *Abutilon* and *Ficus* leaves it was an easy step to those that were mottled by the presence of fungi.

The treatment consisted in immersing the leaves in weak alcohol in glass vessels which stood in the full light of the sun.

The time required for the removal of the chlorophyll depends much upon the thickness and density of the leaf and whether it was freshly gathered or a dried specimen. Usually two or three days are sufficient for the blanching of the leaf when it is ready to be placed in a weak solution of iodine made by adding an ounce of the ordinary solution of iodine in iodide of potassium to a pint of water.

After remaining for a few hours there may be some changes expected, the thinner the leaf the more rapid the reaction. The starch in the leaf responds to the iodine test and, turning blue, brings out the locality where the starch is present in the leaf.

The first subject to be tested chanced to be some dried leaflets of *Potentilla Monspeliensis* affected with the *Peronospora potentillae* DeBy., which, somewhat to my surprise, became quite distinctly blue at the areas where the fungus was bearing its conidia.

This fungus produces angular patches of a brownish color, easily seen from the upper side, while below the surface of the spots become covered with the conidiophores and conidia of the fungus giving this side of the leaf a mildewy appearance in small patches with irregular outlines.

In this instance the leaflets were first placed in 50 per cent. alcohol in a large culture jar with glass cover and set in the sun. After the green has been removed the leaves were transferred to another culture jar containing the iodine and kept in the dark.

* Read before Section G, A. A. A. S., at Boston, August 23, 1898.

In the course of twenty-four hours the portion of the bleached leaflets containing starch were indicated by a darker tinge than elsewhere. A longer exposure to the iodine brought out the starch so that it was located distinctly, and it was found that, with the specimens employed, the starch was confined almost exclusively to the mildewed patches and the neighboring small angular areas that adjoin the portions that already showed the *Peronospora*.

The darkening at these diseased patches was sufficient so that a sun print was taken of a leaflet and afterwards enlarged to five times its natural size, as was shown in a photograph. The original picture was made by placing the leaf directly against the sensitized plate so that every detail is exactly as in the leaf, both as to size and position of all parts.

It is to be remembered that the blanching was not complete, and the iodine, while coloring the starch blue, also tinged the other portions of the tissue of that peculiar "dirty yellow" that is always the bane of the photographer. In short, the picture in this instance, as in all others, is only a faint record of what may be seen when looking at the treated specimen.

One of the most interesting of the observations made under the subject in hand was with *Synchytrium decipiens* Farl. in the leaves of hog-peanut, *Falcata comosa* (L.) Kuntze.

It will be remembered that the Chytridiaceae to which our subject belongs is without hyphae and, therefore, the locality of the fungus is easily determined by that of the infested cells. The leaflets of this host are thin and the iodine easily penetrates the tissue and for the same reason the starch, even when in small amounts, can be distinctly seen.

In the dozen or so of leaves examined it was found that the starch is located immediately around the spore cysts and extends from it through usually one mesh or irregular area made by the ultimate ramifications of the vascular framework. As a matter of fact the fungus is generally located along the lateral veins of the leaf and these are brought out as irregular blue lines when the leaves have been subjected to the iodine test. When a leaflet is quite badly diseased with the *Synchytrium* it will take a blue color throughout all its tissues.

A leaf of *Amarantus retroflexus* L., having seven large circular

patches of *Cystopus bliti* (Biv.) each nearly a third of an inch in diameter was placed in the iodine solution without the previous bleaching, when, after a week, it was found that practically no starch was in the pustular portion, but close around the diseased area was a deep blue circle. All the healthy portion of the leaf contained starch in considerable quantity, but the large amounts in the tissue immediately bordering the portion bearing the conidia of the fungus were in striking contrast with all other parts.

The most striking subject among the leaf parasites is the mandrake leaf infested with *Puccinia podophylli* Schw. It is interesting for the very large amounts of starch that are located in the infested areas, and also for the sharp line of demarcation between the diseased and the healthy portions of the leaf. In other words the *Puccinia* seems to find the ultimate ramifications of the vascular system a complete barrier to its lateral growth, and it seems that the infection in many instances is local and does not reach beyond the angular boundaries made by the smaller veins. The same lines become the enclosures for the starch-bearing areas.

It will also be seen here that the infested areas bear some relationship to the main veins in this, that one side at least of nearly every infested area is against a vein of considerable size, a subject that is a study in itself.

Another fact is noticed in the mandrake, namely, that as a rule there is less starch in the center of the rusted mesh than nearer the periphery.

One of the most striking instances of starch localization is found in the leaves of ordinary corn that are infested with the smut (*Ustilago maydis* DC.). Pieces of leaves that were more or less distorted by nodules and projections of the smut-bearing tissue that had been in alcohol for a year as material for class study of the smut were placed in the iodine, when the blue color began almost immediately to appear in the swollen tissue. All of the bullate portions of the leaves due to the infestation of the fungus finally became of a strong blue color.

A study of the subject with the hand lens showed that, while there was an abundance of starch in the warts, around each center there was a very small space free from starch followed by the

ordinary leaf tissue strongly discolored by the iodine. It was also seen with the lens that the blue color was not uniformly distributed, but in very narrow lines running parallel with the midrib and corresponding with the smaller vascular strands in the leaf.

If we turn now from the consideration of leaves infested with fungi to stems that are similarly attacked the same rule obtains. For example, some hypertrophied flower stalks of cultivated radish that were in stock in formalin as student's material were thinly sectioned and placed in a weak solution of iodine, when, within a minute, the characteristic bluish tinge came into the sections. Similar thin sections were made of the same stems, but at places where no swelling or distortion had taken place and these showed but the smallest amount of starch and that was usually in a single ring of cells located just outside of the bast. As the bast is not continuous the starch-bearing sheath so-to-say was likewise irregular and bent inward at the intervals between the bast stands. There was practically no starch inside of this thin layer, but a small amount outside of it.

In the infested stems the starch is very abundant and in largest amounts in the parenchyma, lying between the wedge-shaped bundles and extending from them throughout the pith. Beside this there is the starch-bearing layer above mentioned, but outside of it there is no starch. The distribution of the oöspores does not seem to bear any relation to that of the starch.

A similar condition of things was met with in the hypertrophied stems (*Exoascus* sp.) of the wild goose plum (*Prunus hortulana* Bailey) when compared with the normal parts of the same twigs.

The galls of the peach roots are simply gorged with starch, and thin sections of them turn to a dark blue color. This color may be brought out by cutting the knot through the middle and shaving the surface smooth and applying iodine. Certain irregular layers and folds will be darker than others, showing that the starch is distributed most abundantly around the wood proper and not in it.

An interesting study in this direction was made of the cedar galls of *Gymnosporangium macropus* Lk., where the starch is packed away in the enlarged host cells to their utmost capacity, and thin sections through the centers of large galls display a neat fan-shaped

appearance after they have been in iodine for a few minutes. The ordinary wood of the gall-bearing twigs show with the same treatment only a small amount of starch, not enough to appear even bluish to the eye after being treated with iodine.

There are certain species of fungi that cause an upright growth of the host when normally the stems are prostrate or reclined. The purslane plants affected with *Cystopus portulacae* (DC.) are a case in point. An examination has been made of the upright, usually dwarfed and badly infested stems of the *Portulaca oleracea* as compared with similar portions of the healthy and prostrate branches. In the latter it is noted that there is much more pink coloring in the sap of the epidermal cells of the upper than the lower side and a larger amount of chlorophyll in the exposed than in the shaded half of the stems as they lie upon the ground. The wood zone is much nearer the outside upon the upper than the lower half of the stem, but there seems to be no marked difference in the wood zone itself, which is circular or oval in outline, consisting of a broken ring of 15 to 20 vascular bundles.

In the healthy stem, while the starch is scattered somewhat, it is confined quite closely to a thin layer of small cells just outside of the ring of wood. The upright mildewed stems have less chlorophyll present, but aside from this the most noticeable difference is the fact that the starch zone is indistinct, the granules being distributed in the cells of the parenchyma both within and outside the starch-bearing sheath so distinctly differentiated in the healthy stem.

In sections made of turnips suffering from the club root fungus, *Plasmodiophora brassicae* Wor., it was quickly observed that the diseased portions were quite generally starch-bearing. Thin sections treated with iodine demonstrated to the eye and the compound microscope that the main portion of the starch is contained in the cells infested with the slime mould. Sections of the same turnip taken in its healthy portions showed but a minimum of starch and this is located in the cells of the cortex. In short, where large slices of the healthy and diseased tissue were laid in weak iodine in a porcelain dish the former showed little or no starch, while the diseased portions appeared almost black.

Thin sections of the large tubercles formed by *Rhizobium*

leguminosarum Fk., were laid in the iodine solution along with those from the roots near by and bearing the galls. The tissue of the tubercles turned blue almost immediately, while the slices of the roots themselves gave only the faintest indications of starch. The tubercle has a layer of loose cellular tissue as a covering inside of which is the denser substance abounding in the bacterioids. It is this covering that is gorged with starch and from it and reaching in towards the center of the gall are irregular anastomosing broad lines, also containing starch.

Plants that are victims to parasitic fungi may possibly be influenced as if they were wounded. Richards, in his extended experiments upon the respiration of wounded plants,* has concluded that there is an increased respiration after an injury to plant tissue, varying in amount and duration with the character of tissue and extent of wound. "This increased respiration," to quote Dr. Richards' words, "may be ascribed to an effort on the part of the plant to recover from the injury by which the ordinary functions of the plants are stimulated, thereby demanding and necessitating an increased supply of oxygen." Bohn, † referred to by Richards, "concludes there is no doubt that the cause of it (respiration) is alone the irritation of the wound itself and not the increased opportunity for the action of the oxygen of the air." But Richards' experiments and those of Stich, whose paper in Flora has not been consulted, show "that the oxygen of the air plays an important part in the reaction of the respiratory function of the plant toward injury."

Townsend ‡ in his extensive studies states that slight injuries accelerate the rate of growth and will continue for several days. He states, "The change in the rate of growth of higher plants under the influence of a single irritation begins gradually, reaches its maximum in from twelve to ninety-six hours and gradually diminishes until the normal rate is resumed." Also "The influence of an irritation due to cutting or other injury is capable of acting through a distance of several hundred millimeters."

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* Annals of Botany, 10: 531–582. D. 1896.

† Ueber die Respiration der Kartoffel. Bot. Zeitung, 45: 671. 1887.

‡ The Correlation of Growth under the Influence of Injuries. Annals of Botany, 11: 509–532. D. 1897.

It is not the purpose, however, of this paper to do more than call attention to the distribution of starch as affected by parasitic fungi. The infesting fungus induces a change in the surrounding tissue, more or less apparent, with the storage therein of food material as shown by the starch test and demonstrated in the instances noted above.

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